

ON THE LARVAL AND PUPAL STAGES OF  
*ANOPHELES MACULIPENNIS*, MEIGEN.

Plates IX and X.

(Continued from *Journ. of Hyg.* Vol. vii. p. 318.)

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PART II. THE LARVA (*continued*).

*The Muscular System.*

*The muscles of the head.* In the preceding part of this paper (*Journ. of Hyg.*, 1907, p. 297) some account was given of the muscles of the pharynx and there remain to be considered the rest of the muscles of the head.

These are concerned with the movements of the brushes, the antennae, the mandibles, the maxillae, and the labium.

The muscles of the brushes consist of two pairs, an external and an internal (Figs. 1 and 2). Both pairs arise from the dorsal wall of the head-capsule, and their points of origin coincide with certain symmetrical patches of pigment situated on the surface of the head. Each pair passes forwards to be inserted into the base of the brush of its side. The external muscles (*b'. m'.*) take their origin between and in front of the eyes, and are separated from one another by an interval equal to about half the diameter of the head at its widest part. They converge somewhat as they pass forwards, and each muscle is attached to a chitinous apodeme of the epipharynx. The internal muscles (*b.m.*) arise almost in contact with one another in the middle line of the head just behind the eyes. For the greater part of their course they run almost parallel with one another, and are likewise inserted into a pair of apodemes at the bases of the brushes, slightly above and internal to those of the external muscles.

With regard to the working of these muscles, so far as could be ascertained, they appeared to contract simultaneously with the result that the brushes are depressed so that they become directed backwards and inwards and lie within the space enclosed by the mouth-parts, and their setae come in contact with the surface of the epipharynx. When the muscles are relaxed the brushes regain their normal attitude in virtue of the elasticity of the chitinous framework at their bases upon which the strain is exerted.

The rapid backward and forward motion of the brushes can be readily observed, and though as a rule they work simultaneously, at times one brush may work independently of its fellow.

A third pair of muscles, the *epipharyngeal muscles*<sup>1</sup> (*ep.m.* in Figs. 1 and 2), arise from the middle line on the roof of the head-capsule and just behind the points of origin of the external muscles of the brushes. They pass forwards very nearly parallel to each other and are inserted close together into the membrane of the epipharynx behind the points of insertion of the muscles of the brushes. They appear to function principally as elevator muscles to the roof of the mouth.

At the base of each antenna, along its inner aspect, an *antennal muscle* (*at.m.* in Fig. 1) is inserted. It takes its origin from a strongly chitinated apodeme (the tentorium) arising from the floor of the head. The muscle passes obliquely upwards and outwards in its course to the antenna.

<sup>1</sup> These muscles have already been referred to in the account of the pharynx (*Journ. of Hyg.* 1907, p. 299).

The remaining muscles of the head which have yet to be dealt with are those belonging to the mouth-parts (Fig. 2), and the most prominent of them are the muscles of the mandibles.

Each mandible is moved by a pair of stout muscles, an adductor, which is attached to its inner angle, and an abductor attached to its outer angle. The adductor muscle is the stouter of the two and consists of four separate bands of fibres (*a.m.*). Three of these arise from the wall of the head-capsule a short distance behind and below the eyes, but the fourth band arises separately at a point somewhat in front of the places of origin of the former, and, moreover, it is ventro-lateral in position. The four bundles pass upwards and inwards, converging as they do so, and are inserted into a stout chitinous process from the mandible. The abductor muscle similarly consists of four distinct bands or bundles of fibres (*a'.m'.*), two of these arise close to the three dorsal bands of the adductor while the other two take their origin near to the ventral bundle of the latter.

Attached to about the middle of the base of each maxilla are a pair of muscle bands (*mx.*). If these be traced backwards they are seen to run in close proximity to each other, and their points of origin are situated ventro-laterally on the cranial wall, near to those of the ventral bands of the abductor muscle of the mandible.

The labial plate is provided with a pair of depressor muscles; they are attached on either side near to the base of that organ where it becomes confluent with the ventral surface of the cranium, and converge as they pass forwards to their points of insertion.

Owing to the imperfect transparency of the head-capsule it has not been possible to fully study the movements of the various muscles in the living larva. Their functions, as implied by the names here given, have been largely inferred from a study of their relations to the different parts principally by means of serial sections.

*The muscles of the thorax.* In the thorax there is a well developed system of longitudinal muscles arranged in a dorsal and a ventral series. The dorsal series, or longitudinal tergal muscles, lie a short distance beneath the integument and occupy much of the mid-dorsal area above the digestive canal. The ventral series, or longitudinal sternal muscles, form two groups, one on either side of the nervous system. Immediately in front of the prothoracic ganglia, a transverse muscle band crosses over the nerve cord and unites together certain of the longitudinal sternal muscles. Tergo-sternal muscles are also present, and the most anterior of them is a stout muscle which takes origin from

the dorsal integument in the prothoracic region on either side. This muscle passes downwards and outwards, and becomes attached to the ventral body wall, just external to the imaginal prothoracic leg-bud of its side. About the middle of the thorax a second series of such muscles are present; they consist of several bundles, widely separated from one another, which pass downwards and inwards to the sternal region. In the hinder part of the thorax a third series of tergo-sternal muscles are present; they pass almost vertically downwards on either side between the salivary glands and the cardiac coeca.

The musculature of the thorax exhibits clear indications of an arrangement into three segments, and it is especially well seen in connection with the longitudinal sternal muscles.

In the fully grown larva the developing imaginal thoracic muscles form very prominent objects; they are disposed in a longitudinal and a vertical series on either side of the body. They are very deeply staining and are in the form of short bundles in which are distributed great numbers of minute nuclei, but they do not exhibit any cross striation. The longitudinal series lie above the larval longitudinal tergal muscles and take the form of parallel cords situated on either side of the middle line. The vertical series are situated immediately external to the salivary glands, and are arranged in an anterior and a posterior group on each side.

A number of muscles are also found in the neck, and their action brings about the remarkable rotatory movements of the head. The smaller of these muscles lie for the most part in the neck itself and pass in an oblique manner from one side to the other. There are, in addition, some much stouter muscles, which arise from the prothoracic region and pass up through the neck to be inserted into the base of the head.

*The musculature of the abdomen.* In the abdomen there is no segmentally repeated system of tergo-sternal muscles, such as is present in many insects. The longitudinal muscles<sup>1</sup>, however, are very well developed and are much stouter than those of the thorax, and it is due to the action of these muscles that the larva performs its vigorous eel-like movements as it swims tail foremost through the water. The longitudinal tergal muscles are grouped together on either side of the dorsal vessel, and the sternal series have similar relations with the

<sup>1</sup> Grandpré and Charmoy remark (p. 33), "Les principaux muscles sont les muscles longitudinaux et circulaires de l'abdomen"; this statement, however, is incorrect as there are no circular muscles present.



nervous system (Fig. 3). In the eighth and ninth segments, the disposition of the longitudinal muscles is much modified, and many of the muscles come to lie nearer the digestive canal.

Just in front of each nerve ganglion, a transverse band crosses the nerve cord, and passes along the line of junction of two adjacent segments (Fig. 22).

In the eighth abdominal segment there is a special system of muscles in connection with the supporting skeleton of the spiracles. In order to attempt an explanation of these muscles it is necessary to briefly consider first the structure of this skeleton. From the posterior region of this segment of the body a median spiracular lobe, as it may be termed, projects backwards and very slightly upwards (Fig. 4). It stands out free from the body, and is supported basally by a special chitinated sclerite (*sc.*), which is prolonged on either side into a prominent plate provided with large backwardly directed teeth (*pl.*). The plates of either side are connected together by means of a transverse chitinous band (*t.b.*) which passes beneath the median spiracular lobe, and serves also to maintain the latter in a position so as to project out freely from the general surface of the body. The lobe itself consists of two thin lateral plates (*c.pl.*) which have their free margins curved upwards and inwards, and a central, oblong plate of dark chitin with a curiously sculptured surface (*m.pl.*). At the apex of the lobe the two lateral plates are joined together by means of a median transverse plate (*t.pl.*). The two spiracles (*sp.*) are situated at the anterior end of the median lobe near to the base of the latter and where it becomes joined to the segment. In front of and between the spiracles is situated a moveable transverse plate standing more or less at right angles with the general surface of the body (*f.pl.*). This plate is attached to a stout hollow peg of very dark chitin (*p.*) which projects beneath the integument slightly into the cavity of the animal. To this same peg is also attached the median plate (*m.pl.*) of the spiracular lobe.

There are three paired sets of muscles in connection with the respiratory apparatus as follows: firstly, a pair of longitudinal muscles (*l.musc.*), which take their origin from the median peg (*p.*), and pass directly backwards to be inserted into the spiracular lobe, in the mid-ventral line near to the apex of that organ. Secondly, a series of muscles (*i.mus.*) arise from the ventro-lateral margin of the eighth abdominal segment and, pursuing a backward and inward course, they converge and pass between the two longitudinal muscles just referred

to. The two series cross one another (Fig. 5) and are inserted into the median plate (*m.pl.*) of the spiracular lobe along its middle line. Thirdly, a pair of stout muscles arise one on either side close to the imaginal bud of the gonapophysis of its side and slightly dorsal to it (*o.mus.*). These muscles pass backwards and upwards in an oblique direction, and just external to the longitudinal muscles, and are inserted on either side of the median area (*m.pl.*).

When the animal dives suddenly down into the water, the upper surface of the spiracular lobe is converted into a kind of cup which retains, as a rule, a bubble of air. The contractions of the two sets of muscles (*i.mus.* and *o.mus.*) depress the floor of the lobe, with the result that the sides of the organ are curved inwards, and brought much closer together, and, furthermore, by the action of the longitudinal muscles (*l.musc.*), the anterior and posterior portions of the organ similarly become drawn closer together, and in this way a temporary cup is formed. When the muscles are relaxed, the organ spreads itself out flat, and forms a plate which helps to support the larva at the surface of the water.

In the ninth abdominal segment, a pair of stout muscles take their origin one on either side of middle line of the tergum, and pass obliquely downwards and backwards to become attached, one on either side, to the base of the skeleton which supports the ventral fan of hairs (Fig. 6). By means of the contractions of this pair of muscles the ventral fan, or "tail-fin," can be moved to one side or the other as occasion may demand and, in this way, it appears that the fan of hairs functions as a kind of rudder which steers the animal as it is swimming. If this analogy be a correct one, the two muscles may be regarded as being comparable with the tiller cords attached to the rudder of a boat.

#### *The Fat-body.*

The fat-body is almost entirely confined to the thorax and the first seven abdominal segments. It consists of a parietal layer situated just beneath the integument, a visceral layer lining the body-cavity, and lying between the longitudinal muscles and the gut-wall, and a peritracheal layer which invests some of the principal tracheal trunks. The limits of distribution of each of these divisions vary according to the age of the larvac, and a certain amount of individual variation is also noticeable among specimens of as near as possible the same age.

The visceral layer of the fat-body (Figs. 3 and 9, *f.b.*), although but a thin sheet of tissue, is very conspicuous, as its cells are loaded with a

mass of minute dark olive coloured granules which are very refractive. It is in this layer that metabolism appears to be most active, for the granules are much less abundant in other parts of the animal. A good deal of the greenish colour of the larva is due to the fat-body shining through the transparent integument. The fat-body cells are much vacuolated, and the cell boundaries are only to be distinguished with considerable difficulty. Their nuclei are for the most part small and inconspicuous, and are irregularly stellate in form. The protoplasm of the cells contains, in addition to the granules just mentioned, a number of clear spherical globules which stain very readily with Orange G. The structure of the fat-body agrees in all respects with Berlese's description of the same tissue in the larva of *Culex spathaepalpis* Rond, and he states that the globules are of an albuminoid nature (1901, p. 98, and Tav. V, Fig. 60).

The *sub-hypodermal cells* have the general characters of fat-body cells and, moreover, their protoplasm contains similar granules. As already stated in the previous part of this paper (*Journ. of Hyg.*, 1907, p. 296) Viallanes concludes that they come under the category of this tissue.

The various areas of the fat-body are not to be regarded as being quite separate and distinct from one another. The divisions here adopted have been used because they appear to be convenient for descriptive purposes, and in certain parts of the body one layer merges into the other. The parietal layer is interrupted at each segment, and in this respect shows a metameric arrangement. The visceral layer, however, is in the form of a continuous sheet passing directly from one segment to another.

#### *The Reproductive Organs.*

The rudiments of the gonads differ in form in the two sexes, the male rudiment being short and somewhat globular, while that of the female is relatively longer and fusiform.

The male rudiment in a larva measuring 2.5 mm.<sup>1</sup> in length (Fig. 14) consists of a germinal region, a long anteriorly directed terminal filament or, so-called Müller's thread, and a slight rudiment of the future generative duct. The organ is situated in the sixth abdominal segment. In larvae measuring 5.5—7.5 mm. long, the proportions of these three regions have undergone considerable altera-

<sup>1</sup> The measurements are taken from the tips of the brushes to the extremity of the abdomen, and do not include the dorsal tuft of setae on the ninth segment.

tion, and a long rudiment of the future reproductive duct has been developed. It extends backwards nearly to the posterior border of the seventh abdominal segment (Fig. 11). The duct, at first, takes the form of a solid cellular strand, but becomes hollowed out at a later period. The terminal filament is embedded in the visceral layer of the fat-body (Fig. 13 *a.*).

In a larva measuring 6.75 mm. long, the female rudiment occupies both the fifth and sixth abdominal segments (Fig. 12), it is more elongated than that of the male of a corresponding age, and shows marked differences in its histological characters. In both sexes the genital rudiment is invested exteriorly by an apparently structureless tunic, and in the later larval periods the central mass of the organ is seen to consist of a number of small cell-clusters, separated from one another by a small amount of interstitial tissue. It is from these cell-clusters that the spermatocytes in the case of the male and the ovarian follicles in the female become developed (Figs. 12 and 13 *b.*).

But little is known concerning the development of the reproductive organs of Diptera, or other Insecta, during the larval period. Among the Brachycera, some observations are given by Weismann (1864, p. 205, Taf. XIV, Figs. 67—72) in the case of *Musca vomitoria* and *Sarcophaga carnaria*. In the Nemocera, the larval gonads are best known for *Chironomus* (Balbiani, 1885, p. 527, and Miall and Hammond, 1900, p. 135), and *Corethra* (Weismann, 1866, p. 99, Taf. VI). Miall and Shelford also give some brief notes on those of *Phalacrocer* (1897, p. 35), and Lécaillon for the female rudiments in *Culex* (1900, p. 96).

In *Chironomus* and *Cecidomyia* it is known that the sexual germs develop at an extremely early period in the embryonic life, being formed as polar cells at the surface of the egg before the blastoderm has been developed. How far this precocious development of the sexual germs is at all general among Insects is at present unknown.

### *The Nervous System.*

The nervous system consists of the brain or cerebral ganglia, and a ventral nerve chain of twelve ganglia (Fig. 23).

The brain alters considerably in shape and in the relations of the parts, during the growth of the larva, especially with regard to the optic and antennary lobes. In the very young larva, the optic lobe is separated from the antennal lobe by a wider area than is the case in



the late larva and, as development progresses, they become as it were drawn closer together.

In its general structure, the brain consists of an outer sheath of ganglion cells, which are thickly massed together in the optic and antennal lobes, and an inner central area consisting for the most part of nerve fibres. Paired nerves are given off supplying the antennae and larval eyes, together with a frontal nerve, which expands into a frontal ganglion lying anterior to the brain and just above the dorsal pharyngeal muscles. The antennal nerve passes obliquely outwards, and enters the imaginal antennal bud where it terminates (Fig. 20). According to Miall and Hammond a similar condition is found in the *Chironomus* larva and in their book on that Dipteron (1900, p. 130) they remark that the imaginal antenna encloses the larval antennary nerve.

The ventral nerve chain comprises the sub-oesophageal ganglion, three thoracic ganglia, and a series of eight ganglia in the abdomen. The sub-oesophageal ganglion innervates the mandibles, the maxillae, and the labium, and is to be regarded as a complex ganglionic mass exhibiting no traces of the primitive ganglia of which it is composed, but its paired structure is noticeable in sections (Fig. 32). Histologically, it consists of an external investment of ganglion cells enclosing a central medullary mass of nerve fibres, which is directly prolonged outwards into the lateral nerves and connectives. The ganglion cells are principally congregated along its anterior, ventral, and lateral borders and are much less numerous on its dorsal aspect. A structureless coat of connective tissues invests the exterior of the ganglion. Posteriorly, a pair of stout nerve cords are given off which pass through the occipital foramen and unite with the first thoracic ganglion. The remaining ventral ganglia have a very similar histological structure, and consist of an outer layer of ganglion cells which enclose an inner core of nerve fibres, and the whole is invested by a sheath or epineurium. The connectives, though they are in reality double, appear to consist of a single cord. The thoracic ganglia are situated close together, the intervening connectives being very short, and the abdominal ganglia are placed anteriorly in their respective segments near to the line of junction with the segment in front (Fig. 22). In the eighth segment the ganglion is larger than those of the preceding segments, it is somewhat triangular in form, and it gives off pairs of nerves which supply both the eighth and last segments. It is therefore to be regarded as a fusion of the two primitive ganglia of

those segments. Each ganglion of the ventral nerve cord gives off a pair of principal nerves to its segment, and they pass outwards between the longitudinal sternal muscles and the integument (Figs. 3 and 22).

A system of transverse nerves is also present; a fine nerve runs along the junction between each pair of segments above the abdominal nerve cord, and each is connected with the ventral ganglia by means of median nerves. Owing to lack of opportunity at present descriptions of this and several other important features in the *Anopheles* larva are postponed for a future paper.

#### *Circulatory System.*

The heart is a muscular tube which extends from the posterior margin of the eighth abdominal segment, and passes forwards through the thorax into the head, where it terminates in the neighbourhood of the brain. In the region of the abdomen it consists of a series of consecutive chambers, each being provided with a pair of lateral ostia or inlets and, associated with them, are the alary muscles. In the thorax the heart is much reduced in diameter, there are no alary muscles, and it forms a narrow well-defined tube which is usually termed in Insects the aorta. The latter extends forwards through the occipital foramen and becomes enclosed just behind the brain in an irregular mass of tissue which forms a kind of supporting collar or *anneau de soutien*.

The abdominal portion of the heart consists of a series of eight successive chambers and it expands somewhat at its posterior extremity, the last chamber forming a slight bulbous enlargement. The intimate connection that exists between the tracheal system and the terminal region of the heart has already been referred to (*Journ. of Hygiene*, 1907, p. 311, Pl. V).

The structure of the wall of the heart is entirely muscular, and consists of flattened cells whose protoplasm mainly takes the form of striated fibrillae, which are disposed more or less parallel to one another (Fig. 30). Both externally and internally, the heart is invested by a delicate transparent membrane which is to be regarded as the sarcolemma of the muscle cells (Fig. 29). The striated fibres encircle the heart posteriorly in a slightly oblique manner, but further forwards they become arranged in a longitudinal direction so that their cut ends are only visible in transverse section (Fig. 15). The nuclei of the cells are situated laterally and form noticeable bulgings in the heart wall

(Figs. 9 and 15)<sup>1</sup>. In a transverse section only two of the cells are visible at any given place: they are crescentic in form and are united with each other along the dorsal and ventral lines.

The lateral inlets or ostia are situated in pairs opposite one another on either side of the heart (Fig. 10). Each ostium is formed by a pair of enlarged cells which surround the aperture and are noticeable in stained preparations by their nuclei.

The alary muscles (*al.m.* in Figs. 9 and 10) are markedly cross-striated: each muscle takes its origin from the integument on either side and, as it approaches the heart, it spreads out fanwise and divides and sub-divides so as to form a network of fine fibrillae. These fibrillae are arranged in the form of a dorsal and ventral sheet (Fig. 9), and in the space or interval left between them are situated the pericardial cells. In addition to the alary muscles, the heart is maintained in position by means of delicate strands of connective tissue placed at different points.

The pericardial cells (Figs. 9 and 10) are placed laterally in relation to the heart, and form a chain of cells arranged in a linear series on either side. The cells are disposed with their long axes parallel with the length of the body, but the chains are not continuous throughout their length, numerous gaps or breaks in the series being present. They extend right through the abdomen to about the middle of the eighth segment. The cells contain several nuclei and their protoplasm is much vacuolated. In *Sayomyia* the pericardial cells were described by Wagner (1835) as "piriform bodies" and later by Dogiel as "apolar nerve cells" (1877, p. 9). In the anterior part of the thorax, and situated close to the heart on either side, is a longitudinal cord of multinucleated cells (Fig. 15). These two cords occupy very much the same position as the pericardial cells in the region of the abdomen and very possibly they are structures of a similar nature.

The aorta is a forward prolongation of the heart through the thorax into the head. It has stout walls, and the nuclei of the cells are disposed with long axes parallel with the length of the tube. No alary muscles are present. The aorta passes forwards between the two dorsally placed cardiac coeca, and lies just over the oesophagus but, as it approaches the occipital foramen, it comes to lie nearer the dorsal integument. Just behind the brain, it becomes supported by an irregular mass of cells which envelops it both ventrally and laterally.

<sup>1</sup> In the larva of *Sayomyia* the nuclei project a long way inwards and were termed the "stalked cells" by Leydig.



This structure is a kind of supporting collar (*anneau de soutien*) and is composed of cells which have no definite outlines and are in some cases vacuolated. It is invested by a connective-tissue coat, and is maintained in position by some delicate muscle fibres arising from the head-capsule. Furthermore, it is also attached by means of fine membranous strands both to the connective-tissue coat of the oesophagus and, a little further forwards, to the commissure uniting the two cerebral ganglia (Figs. 17, 18 and 21). Posteriorly, the organ bifurcates into a pair of cellular prolongations (Fig. 16); while anteriorly it extends a short distance forwards between the cerebral ganglia. A similar structure has been described by Weismann (1864, Pl. VIII, Fig. 8) in the larvae of Muscids and also by Lowne (pp. 88 and 91). It is, furthermore, found in the larvae of the Syrphidae (Kunckel de Herculais, 1875, Pl. XIII, Fig. 5), of the Tachinid *Thrixion* (Pantel, 1898, p. 170) and of *Sayomyia* (Dogiel, 1877, Fig. 1). In the latter it is placed behind the brain as in *Anopheles*, but in *Thrixion* it lies for the most part above the cerebral commissure and in a more forward position. Dogiel states that in front of the collar the aorta divides into two lamellae, one of which extends under the brain towards the eye, and the other is more dorsally situated. At the point where the two lamellae arise the aorta opens freely into the haemocoelic space of the head. In *Anopheles* the further course of the aorta was found to be very difficult to follow, but it seems to divide into two lamellae as in *Sayomyia*, one of which is closely applied to the under surface of the cerebral commissure, and the other appears to be in intimate association with the dorsal surface of the anterior prolongation of the *anneau de soutien*.

The dorsal lamella in *Sayomyia* and *Anopheles* appears to be the homologue of the *gouttière susoesophagienne* described by Pantel in the heart of the larva of the Tachinid genus *Thrixion*.

#### *The Oenocytes.*

Oenocytes are present in the larva of *Anopheles* and belong to two varieties—the large and the small. The large oenocytes (Fig. 26) are segmentally arranged in clusters, and are present in each of the first seven abdominal segments, but were not observed either in the eighth or ninth segments, and they are likewise wanting in the thorax. In each of the segments where they occur, they consist of two pairs of very large cells on either side, which are situated a short distance beneath the hypodermis and towards the posterior margin of the



segment. Each cluster of these cells is ventro-lateral in position. The cells are rounded or somewhat oval in form with a granular and very deeply staining cytoplasm, and each cell is bounded by a clearly defined membranous investment. Numerous large vacuoles are generally present and when viewed in sections they give the cells the appearance of being penetrated by intra-cellular canals. Each oenocyte has a very prominent nucleus containing chromatin strands situated towards the periphery, and a deeply staining nucleolus is also present.

The small oenocytes are very numerous and, moreover, have no definite arrangement though they are sometimes found in pairs (Figs. 22 and 27). They occur just beneath the hypodermis in the neighbourhood of each group of the larger oenocytes, and are mainly situated anterior to the latter, but they also occur in some numbers along the floor of each segment on either side of the nerve cord. They have a similar marked affinity for staining reagents and frequently each contains a clear vacuole (Fig. 22). A nucleus with a central nucleolus is present, and these cells are easily distinguished from the surrounding fat-body by their definite outlines and their staining properties. They occur in the first seven abdominal segments, a few are also present in the eighth but none were to be discovered in the last segment.

The embryonic development of the oenocytes has been studied by Graber, Heymons, Heider, and Wheeler. They arise by a proliferation of the ectoderm just behind the tracheal invaginations, and similar metameric cell-clusters have also been observed by Tichomiroff in the embryo of the "silkworm."

Wielowiejski (1886) was the first to devote special attention to these cells and the name oenocyte is due to him. He pointed out their resemblance to the blood corpuscles, to the fat-body, to the pericardial cells, and to the light-producing tissue of the phosphorescent organs and classified these different elements together under the category of blood tissue. In his paper he describes the oenocytes in various orders of Insects, but unfortunately his account is not accompanied by any figures. Among the Culicidae, he refers briefly to their occurrence in the larvae of *Sayomyia* (*Corethra*) *plumicornis* and *Culex pipiens*. In the former he mentions that they are of two kinds, the large and small oenocytes, and that they are situated between the cells of the outer layer of the fat-body. They are restricted to the abdominal region and are collected together into segmentally repeated groups (1886, pp. 516—17). In the *Culex* larva he describes their occurrence as follows: "Die kleinen Oenocythen liegen auf der der Leibeshöhle

zugewandten Fläche des äusseren Lappens<sup>1</sup> festgeklebt, oftmals bis 10 und mehr auf einem Querschnitte und zeichnen sich durch grosse Tinctionsfähigkeit vor sonstigen aus. Grosse Oenocythen sind auch in Gruppen zu vier bis fünf segmentweise im Abdomen angeordnet, liegen aber eben so wie die kleinen auf der Innenseite des die Leibeshöhle auskleidenden Fettkörperlappens befestigt."

Oenocytes have been discovered in a number of dipterous larvae, and it is extremely probable that they are of general occurrence throughout the order. In *Chironomus* they have been described by Wielowiejski (p. 515) and Miall and Hammond (1900, pp. 40—42). The large oenocytes are restricted to the abdomen, and consist of a group of five very large cells on either side in the first eight segments. The fifth cell in each group lies in front of the cluster of four cells and, moreover, differs from them in containing two nuclei, viz. a large central one and a small one situated at the periphery. The small oenocytes are very numerous and are found towards the ventral surface of the metathoracic and abdominal segments, just beneath the integument. Oenocytes are also present in the larvae of *Tipula oleracea* (Wielowiejski), *Phalacroceru replicata* (Miall and Shelford, 1897, pp. 352), *Simulium* (Vaney, 1202) and other Nemocera.

Among the Brachycera they appear to have been very little studied. Wielowiejski (p. 520) states that in this group of Diptera they present the characters of the large oenocytes of *Chironomus*, and differ chiefly in being more numerous, and in having a more intimate connection with branches of the tracheae. They have been carefully studied, however, by Pantel (1898, p. 210) in the Tachinid, *Thrixion halidayanum*. In the larva of this fly they are collected into a ventral and a lateral group on each side, in the seven posterior segments of the body. Each group contains as a rule 3-6 cells, which are soldered together into a "chapelet." Pantel puts forward the suggestion that possibly one of the two groups in *Thrixion* is the homologue of the binucleated cell in *Chironomus* and that the latter is in reality the rudiment of a colony of uninucleate cells.

Among the Pupipara, according to Berlese (1901, p. 145) in *Melophagus ovinus*, the oenocytes are disposed in metameric groups in the larva but, at the commencement of nymphosis, they multiply and are found in numbers among the cells of the fat-body, and subsequently disappear at the time when the Malpighian tubes are developing.

<sup>1</sup> i.e. the "Fettkörperlappens."

Oenocytes have been found in almost all orders of Insects, but practically nothing is known regarding their function. Wielowiejski believes that they secrete some unknown substance into the blood, and Anglas (p. 405) is of a similar opinion. On the other hand, Pantel concludes from the fact that they absorb methylene blue very rapidly, like the cells of the Malpighian tubes, that their function is that of excretion. Berlese (in *Melophagus*) concludes that they are urinary or excretory cells from the fact that they become free during nymphosis, at a time when the Malpighian tubes are non-functional, and that they work their way among the cells of the fat-body, &c. in order to remove the products of metabolism from them. After a while the oenocytes disappear, and it is at this period that the Malpighian tubes are fully developed.

Koschevnokov (1900) from his studies on the honey bee also concludes that they are urinary cells.

### *The Imaginal Buds.*

The larva of *Anopheles* forms an excellent subject for the study of the imaginal buds since they occur in this type in a very generalised condition. It is, however, beyond the scope of the present paper to deal with this subject in any detail as it needs a much more prolonged study than I have been able to give to it.

Swammerdam appears to have been the earliest observer to correctly interpret certain of the imaginal buds, and he discovered those of the wings and legs. *Culex* was among the types he studied, and since his time Weismann (1866) has studied the imaginal buds of the larva of *Sayomyia*. A few unpublished observations on those of the former genus were made by Hurst, and are mentioned by Miall and Hammond (1892), and Thompson has described the buds of the mouth-parts in that same genus (1905). I am not aware that there are any further accounts which deal with these structures among larval Culicidae.

I. *The imaginal buds of the head.* The largest and most prominent of the head buds are those belonging to the antennae. The *antennal buds* (Figs. 2 and 20) are placed at the bases of the larval antennae, but are not in any way enclosed by the latter and are, moreover, rather deeply situated within the head. In an *Anopheles* larva about three-fifths grown each imaginal antennal bud is a well developed structure and, owing to the growth of the organ within a limited space, the basal joints are somewhat folded and telescoped into one another. As the appendage enlarges, its outer sheath or peripodial membrane



(*pp.m.*) becomes a good deal stretched and is reduced to a thin layer of tissue. At this stage the first three basal joints are already developed but the remainder of the organ is still unsegmented. In a fully grown larva the second joint is greatly enlarged, especially in the male, and its cellular wall is very much thickened so as to almost obliterate the cavity of the joint. This condition is a stage in the development of the prominent antennal sense organ known as Johnston's organ. Although the antennal buds are situated the deepest below the hypodermis of all the imaginal buds, they retain their connection with the surface of the head by means of a short canal which is the persistent mouth of the early invagination.

*The buds of the mouth-parts.* The earliest indications of the formation of the future mouth-parts are noticeable as special thickenings of the hypodermis, situated at localised points beneath the entele of the larval mouth-organs. Of these buds, the rudiments of the future labium, labrum, and maxillary palpi alone attain the condition of imaginal folds prior to pupation. Those of the mandibles and the maxillae remain in the condition of simple, hypodermal thickenings, but are easily recognisable on account of their great affinity for staining reagents. The invaginations of the first mentioned series of buds are superficial in position, and they maintain their communication with the surface by means of wide mouths or openings. The bud of the labrum is unpaired and situated in the middle line. It commences as a hypodermal thickening on the roof of the head and gradually elongates and comes to lie some distance backwards. Those of the maxillary palpi are small structures, and are situated at the bases of the same organs in the larva. The labial bud is extremely prominent (Fig. 31) and takes the form of a paired structure projecting from the bottom of a wide pocket. It is the earliest to appear of the imaginal buds that go to form the mouth-parts. Its first indication in the young larva is in the shape of a thickening of the hypodermis underlying the anterior border of the labial plate. In longitudinal sections through the head of a fully grown larva, the labial buds form a prominent pair of hollow pointed projections which are confluent only at their bases.

II. *The thoracic buds.* In the thoracic region three successive pairs of buds are present on either side belonging to the pro-, meso-, and metathorax respectively. They are disposed in a dorsal and ventral series, the latter eventually giving rise to the three pairs of legs, while of the former series the first pair form the pupal respiratory siphons, the second pair the future wings, and the third pair the halteres. All



the thoracic buds are relatively very large in size, and are superficial in position, lying just beneath the integument. At first they each have the structure of simple buds (Fig. 19) of thickened hypodermis, enclosing a small amount of "mesenchymatous" tissue, but, by the time the larva has become fully grown, they are seen to exhibit a high degree of specialisation. The dorsal prothoracic buds have become the completely formed respiratory trumpets; those of the mesothorax are highly complex organs (Fig. 25), and are much folded and plicated. They already exhibit a wing-like character, the hypodermal cells being greatly drawn out at right angles to the surface of the bud, assuming a pillar-like form, and the upper and lower hypodermal layers meet and fuse with one another. In places they are excavated into hollow tube-like spaces which are forerunners of certain of the future nervures; the dorsal metathoracic buds remain in a more generalised condition, being the smallest and least advanced of the series. The leg-buds are at this period long tube-like organs which are bent upon themselves.

III. *The imaginal buds of the abdomen* comprise a dorsal and ventral pair situated on either side, near the hinder extremity of the body. The dorsal pair of buds form the pupal tail fins; they are placed one on either side of the eighth abdominal segment, and lie within the cavity of the supporting skeleton of the larval spiracles (Figs. 7 and 8). They exhibit very much the same structure as the buds of the wings, the hypodermal cells being enormously drawn out at right angles to the surface of the organ and the protoplasm reduced to narrow strands in which are distributed extremely small nuclei. They are greatly folded, on account of being confined within a limited space, and investing the free surfaces of the folds is a well developed cuticle. They are situated within the lateral plate (*sc.*) of the spiracular skeleton, and project freely backwards reaching to the transverse chitinous band (*tb.*), and thus lying beneath the spiracular lobe. They appear to differ from the other buds in being directly formed by modification of the hypodermis, without the latter being previously invaginated to form a pocket, and from the bottom of which the buds arise usually as finger-like evaginations. On this account, the buds of the pupal fins are not enclosed within an outer wall or peripodial membrane.

The second pair of buds (Fig. 28) are ventral in their position, and placed close to the points of origin of the outer vertical muscles (*o.mus.* in Fig. 5) of the spiracular apparatus. These buds like the rest lie immediately beneath the hypodermis, and maintain a free communication outwards by means of the mouth of the original

invagination. They are destined to form the gonapophyses or accessory copulatory organs of the imago.

The following table shows the general conditions of the imaginal buds among the Diptera, and the relationships of the Culicidae in this respect to other forms.

*Imaginal buds of larval origin.*

A. Imaginal buds superficial in position being situated just below the hypodermis; the primitive invaginations remain permanently open.

1. *Anopheles*; *Culex* (Miall and Hammond ex mss. Hurst). Head folds shallow, not extending into larval thorax.

2. *Corethra* (Weismann); *Simulium* (Weismann, Vaney); *Tanypus* (Vaney). Intermediate between 1 and 3.

3. *Chironomus* (Miall and Hammond); *Ceratopogon* (Miall and Hammond); *Stratiomys* (Vaney)?; *Cecidomyia* (Marchal). Head folds extend into larval thorax.

*Imaginal buds for the most part of embryonic origin.*

B. Thoracic buds superficial in position, cephalic buds as in C.

*Melophagus* (Pratt). Possess an additional pair of ventral cephalic buds not discovered in other Diptera.

C. Imaginal buds deeply situated below hypodermis, only maintaining their connection with the latter by means of a thread-like pedicle. Cephalic buds situated in larval thorax.

*Muscids* (Weismann); *Anthomyia* (Ganin); *Volucella* (Kunckel d'Herculais); *Eristalis* (Wahl); *Gastrophilus* (Vaney).

*The Eyes.*

In newly hatched larvae, and in larvae measuring up to 2.75—3.5 mm. in length, a single oval deeply pigmented eye-spot is present on either side of the head. These *larval eyes* ("Nebenaugen") are placed about half way between the neck and the bases of the antennae, and each has its long axis disposed transversely to the length of the head (*i.e.* in Fig. 24 *a* and *b*). In larvae exceeding that length a second type of eye commences to develop, and is first noticeable in the form of a few small rounded pigmented patches situated above, and slightly behind, the larval eyes. These pigmented areas increase in number with the growth of the larva, and the newly added elements can be distinguished

by their nut-brown colour, whereas the already existing ones, owing to their greater amount of pigment, appear dense black (Fig. 24 *a*). These are the rudiments of the future imaginal compound eye ("Hauptauge"). As development proceeds, each of the small pigmented elements increases in size, with the result that it becomes in close contact with its neighbours, and in a larva about 7 mm. long they collectively form a conspicuous black crescentric shaped tract on either side of the head (Fig. 24 *b*). The subsequent extension of the organ is effected by new elements being added around its periphery.

A short optic nerve is present on either side in relation with the larval eye; in larvae about 7 mm. long a nerve from the imaginal ocular rudiment is distinguishable, and the two nerves of a side combine to form the main optic nerve (*b*. and *c*.).

When viewed in sections, the larval eye consists of a central densely pigmented area bordered by elongated cells whose narrower inner ends are directed towards the centre of the organ (*c*.). The eye is densely loaded with pigment of a rust-brown colour which appears black when viewed in thick layers.

The developing imaginal eye consists of a number of deeply pigmented fusiform bodies, placed at right angles with the surface of the head. These elements are the ommatidia of the compound eye, and they each consist, as seen in transverse section, of a central axis or rhabdom surrounded by a circlet of densely pigmented cells (the retinulae). Intervening between the ommatidia and the external cuticle is a layer of but little modified hypodermal cells (*h*). It is destined to form on the outside the corneal facets of the imaginal eye, and on its inside the crystal cells.

The cells of the hypodermis immediately bordering on the imaginal ocular rudiment are markedly columnar in form, and in places it is folded or invaginated into small pit-like areas, which are the very early stages in the development of new ommatidia.

In addition to the Culicidae, certain other families of nemocerous Diptera, having eucephalous larvae, are remarkable in that the imaginal eye develops in close relation with that of the larva and at an early stage in the larval life-history. A short comparative study of the eyes, in both the larvae and pupae of such Diptera, has recently been made by Zavřel (1907).

*General Remarks on the Larvae of the Culicidae.*

The larvae of the Culicidae may be defined as being aquatic, eucephalous, and with the first three post-cephalic segments fused together to form an evident and greatly enlarged thoracic mass. Although there is but little difficulty in identifying typical Culicid larvae, the greatly enlarged thorax affords the most evident *constant* morphological character, which is common to all the larvae of the family, and by means of which they may be readily separated from those of the allied Nemocerous families Dixidae, Psychodidae, Tipulidae and Chironomidae. It is true that, in the latter family, the first three post-cephalic segments become greatly swollen towards the end of larval life, in consequence of the developing imaginal organs contained within them. In the larval Chironomidae, however, there is no fusion into a compact thorax though the limits between the second and third segments may become partially obliterated. That the thorax of the Culicid larvae is composed of the first three post-cephalic segments is evident from the fact that it contains three of the ventral nerve ganglia, together with three double series of buds of the imaginal thoracic appendages, and moreover its musculature retains to some extent a trisegmental arrangement; externally, however, its composition is not by any means obvious. In *Corethrella brakeleyi* Coq. according to Johannsen (p. 401, pl. 40), the three segments can be perfectly clearly made out, since they have not undergone the same degree of fusion seen among other Culicid larvae. As a rule, the only external indication is to be seen in the principal hairs being arranged in three main transverse series. In all Culicid larvae the abdomen consists of nine segments.

Three principal types of modification are to be seen among Culicid larvae, viz.

(1) The *Anopheles* type where the tracheal system communicates with the exterior by means of a pair of spiracles situated on the dorsal aspect of the eighth abdominal segment, and the respiratory siphon is not developed. Palmate hairs are present on a variable number of the abdominal segments<sup>1</sup>, and the comb scales are replaced on either side by a chitinous plate bearing a series of posterior teeth.

<sup>1</sup> In some species (*A. fluviatilis* and *A. culicifacies*) a pair are present on the thorax and on each of the abdominal segments except the last (James, p. 25).



(2) The *Culex* type, in which the spiracles are situated at the extremity of a siphon formed as a drawing out or prolongation of the dorsal region of the eighth abdominal segment. The two main longitudinal tracheal trunks are usually of a much greater diameter than in the *Anopheles* type, and apparently function to some extent as "air reservoirs." There are no palmate hairs, and the comb almost always consists of a variable number of scale-like spines.

(3) The *Sayomyia* type is, on the whole, the most modified among the Culicidae. The spiracles and respiratory tube are absent and the tracheal system is greatly reduced and mainly consists of two pairs of air vesicles which are situated respectively in the thorax and seventh abdominal segment.

Theobald (1905) recognises eight sub-families among the Culicidae viz. the Anophelinae, Megarhininae, Toxorhynchitinae, Culicinae, Joblotinae, Aedomyinae, Heptaphlebomyinae and Corethrinae.

The *Anophelinae* form a tolerably natural group and exhibit a great similarity among their larvae. The most obvious differences that are to be found are seen in the characters of the frontal, plumose, and palmate hairs, the antennae, the form of the comb and the labial plate.

The *Megarhininae* comprise the single genus *Megarhinus*. The larva is of the *Culex* type, but is remarkable in having the comb represented by a large chitinous plate and in the pecten being absent. It is said to possess a rudimentary spiracle on the last abdominal segment (Felt, 1905, p. 445). The larva of *Toxorhynchites* very closely resembles that of *Megarhinus* and it has been pointed out by Christophers (1906, p. 13) that they agree very closely in the shape of the head and clypeus, in having the brushes modified into "clasping organs" for seizing their prey and in the anal gills being reduced to stump-like papillae.

In *T. immisericors* (Walk.) according to Theobald (vol. iii. p. 118) and Green (1905, p. 159) the siphon is excessively short, and there are no anal gills and the larva rests very nearly parallel to the surface film.

The *Culicinae* exhibit a wide range of variation in their larval structure. The most striking and variable organ is the siphon which differs greatly in its length and general shape among various genera. Stephens and Christophers obtain what they term the "siphonic index" by dividing the length of the tube by its maximum breadth. This index is useful for comparative purposes and appears, on the whole, to

be very constant in certain genera. According to Christophers in *Stegomyia* the siphonic index varies between 1·6 and 2; in *Desvoidea* it is 1·7; in *Culex*, however, it varies very greatly being 4·3 in *Culex fatigans* Wied., while in *C. mimeticus* Noe, according to Theobald's figure (1903, vol. iii. p. 157) it is at least 14·5 on account of its extremely narrow diameter; in *Theobaldia* it is 6, while in *Taeniorhynchus* it is 13. In addition to the characters afforded by the siphon, numerous other structural variations occur, and some of which are proving of great utility in the specific determinations of the various larvae. The general form of the head, the characters of the antennae and the position of insertion of the antennal tuft, the form of the mouth-parts and especially of the labial plate are all of utility in the identification of species. Less useful characters are afforded by the variations in colour (very rarely) and in the form and arrangement of the cephalic and body setae. The anal gills present some degree of variation, being exceptionally long, according to Christophers, in a species of *Stegomyia* which mainly frequents the bottom of the water; in *Desvoidea* they are large and broad (Theobald, vol. iii. pl. XVI), and in most of the known larvae of this sub-family they are well developed, though in *Culex tigripes* Grand. et Charm. and *Acartomyia*, according to Theobald's figures, they appear to be wanting. A wide variation is exhibited in the pecten, or rows of teeth situated on the siphon and, similarly, much variability is seen in the group of scale-like structures which are collectively termed the comb, and situated on either side of the eighth abdominal segment.

Perhaps, the most divergent larvae of the genus *Culex* are those of *C. mimeticus* which, in addition to the features of the siphon already mentioned, are remarkable on account of the large curved pecten spines (Christophers, p. 10), and the cannibalistic species *C. concolor* Robin. Des. and *C. tigripes*. Both of the latter are highly modified, showing but little relationship with the *Culex* larvae and whose affinities are with *Megarhinus* and *Toxorhynchites*. Christophers suggests provisionally the enlargement of the sub-family Megarhininae so as to include, in addition to *Megarhinus*, *Toxorhynchites* (thus doing away with the sub-family this genus represents), *Mucidus*, *Psorophora* together with *Culex concolor* and *C. tigripes*, and possibly *Janthinosoma* and *Lutzia*. All these forms are specially modified for cannibalistic and carnivorous habits in general. Their brushes are transformed into clasping organs for seizing their prey, the mouth-parts are also specially modified and the shape of the head and clypeus is very characteristic.

The anal gills are either reduced to mere stumps or are completely absent, and the attitude of these larvae in relation to the surface film is much more horizontal than is usual among larvae of the Culicinae. The two species, *C. concolor* and *C. tigripes*, Christophers proposes to place in a new genus *Jamesia*, and provided the larval characters are corroborated by sufficiently important differences in the imago, this procedure is fully warranted. Furthermore, the remarkable larva of *C. mimeticus* suggests the possibility that a new genus may be desirable in this instance also.

It seems highly probable that, as our knowledge of the Culicidae becomes more extended, a rational classification of the family will have to be based on a combination of both larval and imaginal characters, and in no other family of Diptera do the larvae apparently exhibit such exceptionally well-defined features, among both genera and species, for this purpose. At present however, until the morphology and ethology of the larvae have been more critically studied and how far any particular modification may be correlated with a certain mode of life, it seems very nearly impossible to discriminate between those characters which have been developed, perhaps recently, by adaptation and those which are to be regarded as phylogenetic or morphological characters. Until this distinction is clearly recognised the application of larval characters for the purposes of classification loses much of its value. Dyar and Knab (1906) have recently contributed an important paper in which they classify the larvae of the New World mosquitoes independently of the imagines. It remains to be seen, however, how far this scheme will fit in with any system of classification founded on imaginal characters.

Of the *Joblotinae* our knowledge of the larval stage is based on a very brief description given by Theobald (1903, vol. iii. p. 334) of the young larva of *Joblotia niveipes* and the later account of Goeldi (1905, p. 120). It appears to be an extremely divergent form with stout blunt antennae and a pair of remarkable "frontal processes." The latter, however, seem to be really the maxillae. There is a short respiratory siphon of very unusual form, but there seem to be no lateral comb.

In the *Aedomyinae* the larval stages are very little known. In *Aedes fuscus* Osten-Sac. the larva is of the general *Culex* type and possesses four narrow lanceolate gills and a very short siphon (Dyar, 1902, p. 197). According to Felt (1904, p. 340) it so nearly resembles the larvae of *C. Sylvestris* Thed. and *C. impiger* Walk. that it



is difficult to separate them. The larva of *A. smithii* Coq. differs, however, so greatly from that of *A. fuscus* that the systematist if he were relying solely on larval characters would unhesitatingly place it in a separate genus. According to Dyar (1901, p. 178) it possesses but a single pair of anal gills which are broad and rounded at their apices; in the number of its gills it seems to be unique among Culicid larvae and Dyar and Knab regard the upper pair as having been aborted. The mandibles have but a single bristle or curved spine at the apex and furthermore the pecten is entirely wanting. From *A. fuscus* it further differs in the form of the thorax and siphon.

In *Uranotaenia* the larva closely resembles that of a typical *Culex* but the comb is remarkable in that it consists of eight simple spine-like processes attached to the posterior margin of a lateral plate of chitin, and recalls the comb plate of the Anophelinae (Dyar, 1901, p. 179; Felt, 1904, p. 343). The larva of *Deinocerites* is only known from very brief notes given by Theobald (1903, vol. iii., p. 280) and it is not possible to compare it with other forms. His account is also rather obscure, since he confuses the thorax partly with the first two abdominal segments.

The *Heptaphlebomyiinae* include but a single genus whose larva is unknown.

In the *Corethrinae* the larval stages are known for all the described genera. That of *Sayomyia*<sup>1</sup> represents the extreme type of larval development met with in this sub-family. It is almost perfectly transparent, the extremities of the jaws and the pigmented eyes and air reservoirs being the only parts that catch the unaided eye. The fore part of the head is much prolonged, carrying the antennae at the extremity. The pharynx is modified into an eversible tube, and there are remarkable posterior hooks carried on the last abdominal segment. Respiration appears to be almost entirely cutaneous, though a greatly reduced and much modified tracheal system is present. It consists of a pair of longitudinal trunks extending through the greater part of the animal, but does not contain air, except in the thorax and the seventh abdominal segment. In these positions, however, there are situated a pair of pigmented air reservoirs. There are no traces of spiracles present. Situated on the ninth segment of the abdomen are two pairs of anal gills.

The larva of *Corethra*<sup>2</sup> forms a connecting link between that of

<sup>1</sup> *Sayomyia* Coquill. (*Corethra* Loew).

<sup>2</sup> *Corethra* Meigen (*Mochlonyx* Loew).



*Sayomyia* and a typical Culicid larva. The intermediate condition is especially well indicated in the tracheal system which, although it has undergone some amount of reduction, is much better developed than in *Sayomyia* and, moreover, spiracles are present. They are situated at the extremity of a short respiratory siphon arising from the eighth abdominal segment. *Corethra* resembles *Sayomyia* in the form of the antennae but the head is not prolonged anteriorly (Meinert, Tab. II). The larva of *C. Karnerensis* is remarkable in having the extremity of the last segment bordered by a whorl of numerous, short, recurved fleshy processes (Felt, 1904, p. 353).

The larvae of both *Eucorethra* and *Corethrella* resemble *Corethra* much more closely than *Sayomyia*. Both possess a well developed tracheal system resembling that of *Culex* and opening at the extremity of a short siphon. The antennae in *Eucorethra* resemble those of the two preceding genera while those of *Corethrella* are curiously folded back against the head (Felt).

*Pelorempis* is a recently discovered form and very imperfectly known. It resembles *Sayomyia* and *Corethra* in the elongated antennae which are provided with stout spines set at an angle with their long axes. It has the general shape of the head of *Corethra* but the maxillae rather resemble those of *Sayomyia*. In its respiratory system it comes closer to the Anophelinae than to any other of the Culicidae. There is no siphon and the spiracles are supported by a chitinous skeleton resembling very closely that of *Anopheles* (Johannsen, p. 404). *Pelorempis* seems, therefore, to be a synthetic type as regards its larval characters, since it exhibits features belonging to both Corethrinae and Anophelinae.

The relationships of the larvae of the Corethrinae appear on the whole to come nearest to the Culicinae. The fact that they are predaceous, feeding on larvae of their own and other species, and small Entomostraca, &c., might suggest the possibility of their having arisen from the Megarhininae (*sensu latiore* of Christophers) which are similarly carnivorous (and cannibalistic). Such a view, however, would not be tenable since, in the Corethrinae, the antennae are the organs which are specially modified for seizing the prey, while in the latter group, it is the feeding brushes that have become modified for the same purpose.

Rondani in 1856 appears to have been the earliest writer to separate the Corethrinae from the rest of the Culicidae and elevate them to family rank. More recently Coquillett and others have also

advocated this classification. Great stress has been laid on the reduced condition of the mouth-parts which are not adapted for piercing and, correlated with it, the feeding habits of the female. Additional characters have also been found in the venuration of the wings and the absence of scales from the latter. It is the belief of the author of this paper, however, that the Corethrinae are best retained as a sub-family of the Culicidae.

The reasons for this conclusion are as follows:—

The newly discovered genus *Pelorempis*, though agreeing with *Sayomyia* and *Corethra* in the form of the proboscis, resembles the Culicinae in possessing scales to the wings. The larva, moreover, as has already been pointed out, greatly differs from those of the remaining Corethrinae. The *Sayomyia* larva, it is true, is totally different from any other Culicid larva, but those of the remaining Corethrinae are clearly intermediate in the structure between that genus and the general *Culex* type of larva. It is, therefore, clear that the limits of the Corethrinae cannot at present be very well defined and, for that reason, it is better to retain the group as a sub-family only.

The resemblances between the larvae of the Anophelinae and the Culicinae, as regards their internal anatomy (the presence of cardiac coeca and five Malpighian tubes), and the great divergence between those groups and the genus *Sayomyia* (four Malpighian tubes and the absence of cardiac coeca), are sufficient to suggest that an anatomical study, of typical representatives of the different groups, may prove a valuable aid towards a rational classification of the family.

## LITERATURE.

- ANGLAS, J. (1901), Observations sur les métamorphoses internes de l'Abeille. *Bull. Sci. de la France et de la Belg.* t. XXXIV. p. 363.
- BALBIANI, E. G. (1885), Contribution à l'étude de la formation des organes sexuels chez les Insectes. *Rec. Zool. Suisse*, t. II. p. 527.
- BERLESE, A. (1901), Osservazioni sopra fenomeni che avvengono durante la nimfosi degli insetti metabolici. *Rev. di Patolog. Veget.* vol. VIII. p. 1.
- CHRISTOPHERS, S. R. (1906), On the importance of larval characters in the Classification of Mosquitoes. *Sci. Mem. by Officers of the Med. and Sanit. Departs. Govt. India*, N.S. no. 25, Calcutta (office of the Superintendent of Government Printing).
- COQUILLETT, D. W. (1903), A new Culicid genus allied to *Corethra*. *Canad. Entom.* vol. XXXV. p. 189.
- (1906), A Classification of the Mosquitoes of North and Middle America. *U. S. Dept. Agric., Bureau of Entom., Tech. Ser.* no. 11, p. 31.

- DOGIEL, J. (1877), Anatomie u. Physiologie des Herzens der Larve von *Corethra plumicornis*. *Mem. de l'Acad. Sci. St. Pétersb.* 7<sup>me</sup> ser. t. xxiv. no. 10.
- DYAR, H. G. (1902), Illustrations of the larvae of North American Culicidae. *Journ. New York Entom. Soc.* vol. x. p. 194.
- DYAR, H. G. and KNAB F. (1906), The Larvae of the Culicidae classified as independent organisms. *Journ. New York Entom. Soc.* vol. xiv. no. 4, p. 169.
- FELT, E. P. (1904), Mosquitoes or Culicidae of New York State. *N. Y. State Mus. Bull.* 79, Entom. 22. p. 241.
- (1905), Studies in Culicidae; 20th Rep. State Entom. on Injurious and other Insects of the State of N. Y. 1904. *N. Y. State Mus. Bull.* 97, Entom. 24, p. 442.
- GOELDI, E. A. (1905), Os Mosquitos no Pará. *Mem. Mus. Goeldi*, iv. p. 154.
- GRANDPRÉ, A. D. DE, and CHARMOY, D. d'E. DE (1900), *Les Moustiques. Anatomie et Biologie*. Port Louis, Ile Maurice, pp. 59, 5 planches.
- GREEN, E. E. (1905), On *Toxorhynchites immisericors* (Walker), the Elephant Mosquito. *Spolia Zeylanica*, vol. II. pt. 8, p. 159.
- HILL, E. and HAYDON, L. G. (1907), A Contribution to the study of the Characteristics of Larvae of species of Anophelina in South Africa. *Ann. Natal Govt. Mus.*, vol. I. pt. 2, p. 111.
- JAMES, S. P. (1902), Malaria in India. *Sci. Mem. by Officers of the Med. and Sanit. Departs. Govt. India*, N.S. no. 2, Calcutta (office of the Superintendent of Government Printing).
- JOHANNSEN, O. A. (1903), Aquatic Nematoceros Diptera. *New York State Mus. Bull.* 68, Entom. 18, p. 328.
- KOSCHEVNIKOV, G. A. (1900), Ueber den Fettkörper und die Oenocyten der Honigbiene. *Zool. Anz.*, vol. xxiii. no. 618, p. 337.
- KULAGIN, N. (1905), Der Kopfbau bei *Culex* und *Anopheles*. *Zeitschr. für wiss. Zool.* vol. LXXXIII. p. 285.
- KUNCKEL D'HERCULAIS, J. (1875), *Recherches sur l'organisation des Volucelles*. Paris.
- LÉCAILLON, A. (1900), Recherches sur la structure et le développement postembryonnaire de l'ovaire des Insectes—1° *Culex pipiens* L. *Bull. Soc. Entom. Fr.* p. 94.
- LOWNE, B. T. (1890—92), *The Blow Fly*, vol. I. London.
- METALNIKOFF, S. (1902), Beiträge zur Anatomie und Physiologie der Mückenlarve. *Bull. de l'Acad. Sci. St. Pétersb.* (5) xvii. p. 49.
- MIALI, L. C. and HAMMOND, A. R. (1892), The development of the head of the imago of *Chironomus*. *Trans. Linn. Soc.* 2nd ser., v. p. 265.
- (1900), *The Structure and Life-history of the Harlequin Fly*. Oxford: at the Clarendon Press.
- MIALI, L. C. and SHELFORD, R. (1897), The Structure and Life-history of *Phalacroceru replicata*. *Trans. Entom. Soc. Lond.* p. 343.
- PANTEL, J. (1896), *Thrixion halidayanum* Rond. Essai monographique sur les caractères extérieurs, la biologie et l'anatomie d'une larve parasite du groupe des Tachinaires. *La Cellule*, t. xv. p. 1.
- THEOBALD, F. V. (1905), Family Culicidae, in *Genera Insectorum*, edited by P. Wytsman, Bruxelles.

- THEOBALD, F. V. (1901), *Monograph of the Culicidae of the World*, vol. I. London.  
 — (1903), Do. vol. III.  
 THOMPSON, M. T. (1905), Alimentary Canal of the mosquito. *Proc. Boston Soc. Nat. Hist.* vol. XXXII. no. 6, p. 145.  
 VANEY, C. (1902), Contributions à l'étude des larves et les métamorphoses des Diptères. *Ann. de l'Univ. de Lyon*, N. S. I. Sci. med., fasc. 9, p. 178.  
 WEISMANN, A. (1864), *Die Entwicklung der Dipteren*. Leipzig, W. Engelmann.  
 — (1886), Die metamorphose der *Corethra plumicornis*. *Zeitschr. für wiss. Zool.* vol. XVI. p. 45.  
 WAGNER, R. (1835), Ueber Blutkörperchen bei Regenwürmern, Blutegeln und Dipterenlarven. *Müller's Archiv*, p. 311.  
 WIELOWIEJSKI, H. R. von (1886), Über das Blutgewebe der Insekten. *Zeitschr. für wiss. Zool.* vol. XLIII. p. 512.  
 ZAVŘEL J. (1907), Die Augen einiger Dipterenlarven und Puppen. *Zool. Anz.* vol XXXI. p. 247.

## REFERENCE LETTERING.

<i>abd.</i> 5, 6, 8, 9.	Fifth, sixth, eighth and ninth abdominal segments.
<i>abd. g.</i> 1.	First abdominal nerve ganglion.
<i>abd. g.</i> 8 + 9.	Ganglion formed by the concrescence of those of the eight and ninth abdominal segments.
<i>al. m.</i>	Alary muscle.
<i>a. m.</i>	Adductor muscle of mandible.
<i>a<sup>l</sup>. m<sup>l</sup>.</i>	Abductor muscle of mandible.
<i>a. n.</i>	Antennal nerve.
<i>ao.</i>	Aorta.
<i>ap.</i>	Aperture of salivary duct.
<i>at. d.</i>	Imaginal bud of antenna.
<i>at. m.</i>	Antennal muscle.
<i>b.</i>	Brush.
<i>b. m.</i>	Inner retractor muscle of brush.
<i>b<sup>l</sup>. m<sup>l</sup>.</i>	Outer     „     „     „     „
<i>c.</i>	Cuticle.
<i>e. e.</i>	Lateral cellular cord.
<i>e. com.</i>	Cerebral commissure.
<i>d.</i>	Rudiment of generative duct.
<i>d. tr.</i>	Main longitudinal tracheal trunk.
<i>d. v.</i>	Dorsal vessel.
<i>e.</i>	Eye.
<i>ep. m.</i>	Epipharyngeal muscles.
<i>f. b.</i>	Fat-body.
<i>f. n.</i>	Frontal nerve.
<i>f. pl.</i>	Fan-like plate.
<i>g.</i>	Abdominal nerve ganglion.
<i>g. d.</i>	Imaginal buds of gonapophyses.
<i>g. l.</i>	Ganglionic layer.



<i>h.</i>	Hypodermis.
<i>i. mus.</i>	Inner vertical muscle of spiracular lobe.
<i>l. d.</i>	Imaginal bud of left middle leg.
<i>l<sup>1</sup>. d<sup>1</sup>.</i>	Imaginal bud of labium ; left of the paired elements (section somewhat oblique).
<i>l. e.</i>	Larval eye.
<i>l. musc.</i>	Longitudinal muscles of spiracular lobe.
<i>l. pl.</i>	Lateral plate of spiracular lobe.
<i>l. t. m.</i>	Longitudinal tergal muscles.
<i>m.</i>	Muscle attached to supporting collar of heart.
<i>m. b.</i>	Striated muscle bands (in section).
<i>m. c.</i>	Mother cells.
<i>m. g.</i>	Mid-gut.
<i>m. n.</i>	Median nerve.
<i>m. pl.</i>	Median plate of spiracular lobe.
<i>m. s.</i>	"Mesenchyme."
<i>m. x.</i>	Muscle of maxilla.
<i>ner.</i>	Lateral nerve.
<i>o.</i>	Ommatidia.
<i>oen.</i>	Oenocytes (smaller).
<i>o. mus.</i>	Outer vertical muscles of spiracular lobe.
<i>o. n.</i>	Optic nerve.
<i>p.</i>	Chitinous peg.
<i>p. c.</i>	Pericardial cells.
<i>ph.</i>	Pharynx.
<i>ph. w.</i>	Wall of pharynx.
<i>pl.</i>	Lateral toothed plate.
<i>p. m.</i>	Peritrophic membrane.
<i>p. s.</i>	Peripodial space.
<i>r.</i>	Rectum.
<i>sc.</i>	Basal supporting plate of spiracular skeleton.
<i>s. col.</i>	Supporting collar of heart ( <i>anneau de soutien</i> ).
<i>s. col. p.</i>	Posteriorly directed prolongations of the supporting collar.
<i>s. d.</i>	Median salivary duct.
<i>s. g.</i>	Sub-oesophageal ganglion.
<i>sp.</i>	Spiracles.
<i>sup. g.</i>	Brain.
<i>t.</i>	Tracheal trunk to anal gills of its side.
<i>t. b.</i>	Transverse chitinous band.
<i>t<sup>1</sup>. b<sup>1</sup>.</i>	Transverse intersegmental band.
<i>t. f.</i>	Larval "tail-fin."
<i>t. f. m.</i>	Muscles of larval "tail-fin."
<i>t<sup>1</sup>. f<sup>1</sup>.</i>	Terminal filament.
<i>t. f. d.</i>	Imaginal bud of pupal "tail-fin."
<i>th. g. i.</i>	Prothoracic ganglion.
<i>t. pl.</i>	Terminal plate of spiracular lobe.
<i>tr.</i>	Trachea.
<i>w. d.</i>	Imaginal bud of wing.
<i>x - - - y</i>	Direction of the transverse section Fig. 2.

## DESCRIPTION OF THE PLATES.

## PLATE IX.

- Fig. 1. Dorsal aspect of the head of a larva of *Anopheles maculipennis* showing certain of the muscles.
- Fig. 2. Transverse section through the head across the line  $x-y$  in the preceding figure.
- Fig. 3. Typical transverse section through the abdomen and passing through one of the ventral nerve ganglia.
- Fig. 4. Lateral view of the spiracles and their supporting skeleton.
- Fig. 5. Transverse section through the eighth abdominal segment together with the spiracular lobe. (Reconstruction from several sections.)
- Fig. 6. Transverse section through the ninth abdominal segment showing the muscles of the "tail-fin." (Reconstruction from several sections.)
- Fig. 7. Longitudinal and horizontal section through the spiracular lobe passing some distance below the level of the spiracles.
- Fig. 8. Do. passing just beneath the spiracles.
- Fig. 9. Transverse section through the heart and associated structures; 6th abdominal segment.
- Fig. 10. Dorsal aspect of a portion of the heart showing alary muscles and pericardial cells; 6th abdominal segment.
- Fig. 11. Rudiment of the male gonad from larva measuring 6.5 mm. long.
- Fig. 12. Rudiment of the female gonad from a larva measuring 6.75 mm. long. The numbers in this and the preceding figure represent the respective segments in which the organ lies, and the horizontal lines indicate the boundaries between those segments.
- Fig. 13. Transverse sections through three separate regions of the male gonad of a larva about 6 mm. long: (a) through the base of the terminal filament; (b) through the middle of the gonad; (c) through the developing duct.
- Fig. 14. Outline figure of the rudiment of the male gonad in a larva 2.3 mm. in length.
- Fig. 15. Transverse section through the heart and associated cellular cords; anterior part of the thorax.
- Fig. 16. Do. through the heart and posterior prolongations of the supporting collar; commencement of oesophagus.
- Fig. 17. Do. through the middle of the supporting collar together with the heart.
- Fig. 18. Do. through the cerebral commissure and the anterior termination of the heart.
- Fig. 19. Section of imaginal buds of wing and second leg of left side at an early stage in development.
- Fig. 20. Section through imaginal antennal bud and passing through the opening of the primitive invagination.
- Fig. 21. Longitudinal and vertical section passing through the cerebral commissure together with the terminal portion of the heart and the associated supporting collar.









